

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C07C 275/28, C07D 213/06, A61K 31/17, 31/415</b>		A1	(11) International Publication Number: <b>WO 99/00357</b> (43) International Publication Date: <b>7 January 1999 (07.01.99)</b>
(21) International Application Number: <b>PCT/US98/13496</b>		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: <b>29 June 1998 (29.06.98)</b>			
(30) Priority Data: <b>08/884,160</b> 27 June 1997 (27.06.97) US			
(71) Applicant: VERTEX PHARMACEUTICALS INCORPORATED [US/US]; 130 Waverly Street, Cambridge, MA 02139-4242 (US).			
(72) Inventors: SALITURO, Francesco, Gerald; 25 Baker Drive, Marlborough, MA 01752 (US). BEMIS, Guy, W.; 256 Appleton Street, Arlington, MA 01574 (US). GREEN, Jeremy; 21 Greystone, Burlington, MA 01803 (US). KOFRON, James, L.; 21715 31st Street, Bristol, WI 53104 (US).		Published <i>With international search report.</i>	
(74) Agent: HALEY, James, F., Jr.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020 (US).			
(54) Title: INHIBITORS OF p38			
(57) Abstract			
<p>The present invention relates to inhibitors of p38, a mammalian protein kinase involved in cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INHIBITORS OF p38TECHNICAL FIELD OF INVENTION

10        The present invention relates to inhibitors of p38, a mammalian protein kinase involved cell proliferation, cell death and response to extracellular stimuli. The invention also relates to methods for producing these inhibitors. The invention also  
15        provides pharmaceutical compositions comprising the inhibitors of the invention and methods of utilizing those compositions in the treatment and prevention of various disorders.

20        BACKGROUND OF THE INVENTION

Protein kinases are involved in various cellular responses to extracellular signals. Recently, a family of mitogen-activated protein kinases (MAPK) 25 have been discovered. Members of this family are Ser/Thr kinases that activate their substrates by phosphorylation [B. Stein et al., Ann. Rep. Med. Chem., 31, pp. 289-98 (1996)]. MAPKs are themselves activated by a variety of signals including growth factors, 30 cytokines, UV radiation, and stress-inducing agents.

One particularly interesting MAPK is p38. p38, also known as cytokine suppressive anti-inflammatory drug binding protein (CSBP) and RK, was isolated from murine pre-B cells that were transfected 35 with the lipopolysaccharide (LPS) receptor CD14 and induced with LPS. p38 has since been isolated and

-2-

sequenced, as has the cDNA encoding it in humans and mouse. Activation of p38 has been observed in cells stimulated by stresses, such as treatment of 5 lipopolysaccharides (LPS), UV, anisomycin, or osmotic shock, and by cytokines, such as IL-1 and TNF.

Inhibition of p38 kinase leads to a blockade on the production of both IL-1 and TNF. IL-1 and TNF stimulate the production of other proinflammatory cytokines such as IL-6 and IL-8 and have been 10 implicated in acute and chronic inflammatory diseases and in post-menopausal osteoporosis [R. B. Kimble et al., Endocrinol., 136, pp. 3054-61 (1995)].

Based upon this finding it is believed that 15 p38, along with other MAPKs, have a role in mediating cellular response to inflammatory stimuli, such as leukocyte accumulation, macrophage/monocyte activation, tissue resorption, fever, acute phase responses and neutrophilia. In addition, MAPKs, such as p38, have been implicated in cancer, thrombin-induced platelet 20 aggregation, immunodeficiency disorders, autoimmune diseases, cell death, allergies, osteoporosis and neurodegenerative disorders. Inhibitors of p38 have also been implicated in the area of pain management 25 through inhibition of prostaglandin endoperoxide synthase-2 induction. Other diseases associated with IL-1, IL-6, IL-8 or TNF overproduction are set forth in WO 96/21654.

Others have already begun trying to develop 30 drugs that specifically inhibit MAPKs. For example, PCT publication WO 95/31451 describes pyrazole compounds that inhibit MAPKs, and in particular p38. However, the efficacy of these inhibitors *in vivo* is still being investigated.

Accordingly, there is still a great need to 35 develop other potent, p38-specific inhibitors that are

-3-

useful in treating various conditions associated with p38 activation.

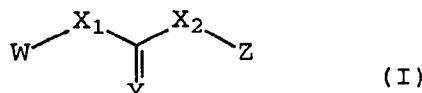
SUMMARY OF THE INVENTION

5

The present invention solves this problem by providing compounds which demonstrate strong and specific inhibition of p38.

These compounds have the general formula:

10



15

wherein:

W is a saturated, partially saturated or aromatic monocyclic or bicyclic ring system containing 0-4 heteroatoms selected from N, O, and S, wherein W 20 optionally comprises up to 4 substituents independently selected from R<sup>1</sup> and R<sup>4</sup>;

wherein R<sup>1</sup> is halogen, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>, CO<sub>2</sub>R<sup>3</sup>, CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-25 methyleneoxy, 1,2-ethylenedioxy or CF<sub>3</sub>;

Y is O, S or NH;

X<sub>1</sub> and X<sub>2</sub> are independently selected from O, S or NR<sup>2</sup>;

wherein R<sup>2</sup> is selected from H or C<sub>1</sub>-C<sub>6</sub> 30 straight or branched alkyl, C<sub>2</sub>-C<sub>6</sub> straight or branched alkenyl or alkynyl, wherein R<sup>2</sup> is optionally substituted with -OH,

-4-

$-N(R^3)_2$ ,  $-Z$ ,  $-CO_2R^3$  or  $-CO-N(R^3)_2$ ;

$R^3$  is selected from H,  $C_1-C_6$  straight or branched alkyl,  $C_2-C_6$  straight or branched alkenyl or alkynyl, or  $C_{6-20}$  aryl wherein  $R^3$  optionally contains up to 4 substituents selected from halo,  $-OH$ ,  $-OR^4$ ,  $-NO_2$ ,  $-NH_2$ ,  $-N(R^4)_2$ ,  $-CO_2R^4$ ,  $-CO-N(R^4)_2$ ,  $-Z$ ,  $-CN$ ,  $-SR^4$ ,  $CF_3$  or  $-SO_2NR^4$ ;

$R^4$  is independently H,  $(C_1-C_6)$ -straight or branched alkyl,  $(C_2-C_6)$ -straight or branched alkenyl or alkynyl;

$Z$  is selected from  $C_3-C_7$ -cycloalkyl,  $C_5-C_7$ -cycloalkenyl or aromatic or non-aromatic 5-7 membered monocyclic or bicyclic ring containing 0-4 heteroatoms selected from N, O and S, wherein  $Z$  optionally comprises up to 4 substituents independently selected from  $R^1$  and  $R^4$ .

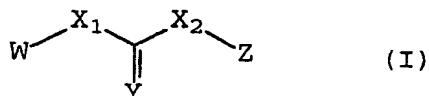
In another embodiment, the invention provides pharmaceutical compositions comprising the p38 inhibitors of this invention. These compositions may be utilized in methods for treating or preventing a variety of disorders, such as cancer, inflammatory diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, viral diseases and neurodegenerative diseases. These compositions are also useful in methods for preventing cell death and hyperplasia and therefore may be used to treat or prevent reperfusion/ischemia in stroke, heart attacks, organ hypoxia. The compositions are also useful in methods for preventing thrombin-induced platelet aggregation. Each of these above-described methods is also part of the present invention.

-5-

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides inhibitors of p38 having the general formula:

5



10 wherein W is a saturated, partially saturated or an aromatic monocyclic or bicyclic ring system containing 0-4 heteroatoms independently selected from N, O, and S, wherein W optionally comprises up to 4 substituents independently selected from R<sup>1</sup> and R<sup>4</sup>.

15 R<sup>1</sup> is selected from halogen, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>, CO<sub>2</sub>R<sup>3</sup>, CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-methylenoxy, 1,2-ethylenedioxy or CF<sub>3</sub>.

Y is O, S or NH.

20 X<sub>1</sub> and X<sub>2</sub> are independently selected from O, S or NR<sup>2</sup>.

wherein R<sup>2</sup> is selected from H or C<sub>1</sub>-C<sub>6</sub> straight or branched alkyl, C<sub>2</sub>-C<sub>6</sub> straight or branched alkenyl or alkynyl, wherein R<sup>2</sup> is optionally substituted with -OH, -N(R<sup>3</sup>)<sub>2</sub>, -Z, -CO<sub>2</sub>R<sup>3</sup> or -CO-N(R<sup>3</sup>)<sub>2</sub>.

25 R<sup>3</sup> is selected from H, C<sub>1</sub>-C<sub>6</sub> straight or branched alkyl, C<sub>2</sub>-C<sub>6</sub> straight or branched alkenyl or alkynyl or C<sub>6</sub>-20 aryl, wherein R<sup>3</sup> optionally contains up to 4 substituents selected from halo, -OH, -OR<sup>4</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -N(R<sup>4</sup>)<sub>2</sub>, -CO<sub>2</sub>R<sup>4</sup>, -CO-N(R<sup>4</sup>)<sub>2</sub>, -Z, -CN, -SR<sup>4</sup>, CF<sub>3</sub> or

-6-

-SO<sub>2</sub>NR<sup>4</sup>.

R<sup>4</sup> is independently H, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl.

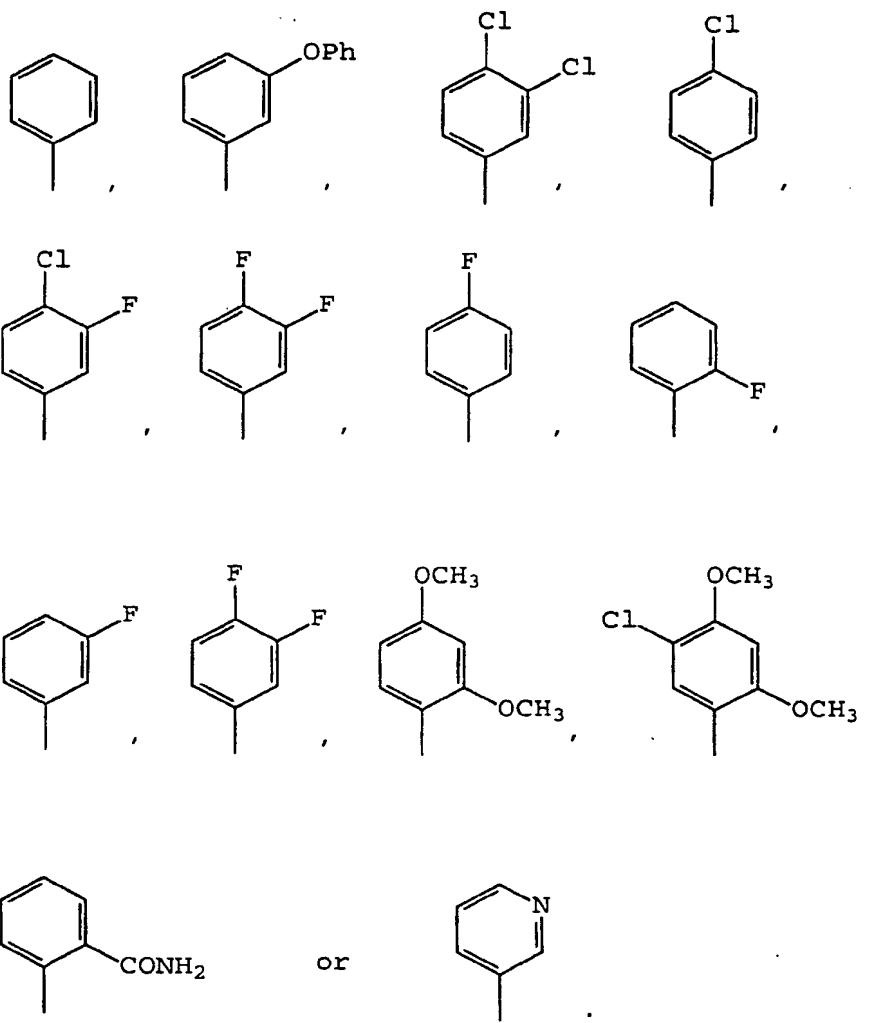
5           Z is selected from C<sub>3</sub>-C<sub>7</sub>-cycloalkyl, C<sub>5</sub>-C<sub>7</sub>-cycloalkenyl or aromatic or non-aromatic 5-7 membered monocyclic or bicyclic ring systems containing 0-4 heteroatoms selected from N, O and S, wherein Z optionally comprises up to 4 substituents independently  
10           selected from R<sup>1</sup> and R<sup>4</sup>.

According to a preferred embodiment, W is an aromatic or non-aromatic 5-7 membered monocyclic ring containing up to 3 heteroatoms selected from O, S and N, and optionally containing up to 3 substituents  
15           selected from halo, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>, CO<sub>2</sub>R<sup>3</sup>, CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-methylenoxy, 1,2-ethylenedioxy, CF<sub>3</sub>, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl.

20           According to a more preferred embodiment, W phenyl or pyridyl, each containing up to 3 substituents selected from halo, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>, CO<sub>2</sub>R<sup>3</sup>, CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-methylenoxy, 1,2-ethylenedioxy, CF<sub>3</sub> or (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl.  
25

-7-

Some specific examples of the preferred W are:



5

Most preferably, W is phenyl, 3,4-dichlorophenyl, 2-fluorophenyl or 2-amidophenyl.

According to a preferred embodiment, Z is a 5-7 membered aromatic or non aromatic ring system, 10 optionally containing up to 4 heteroatoms independently selected from N, O and S, wherein Z optionally

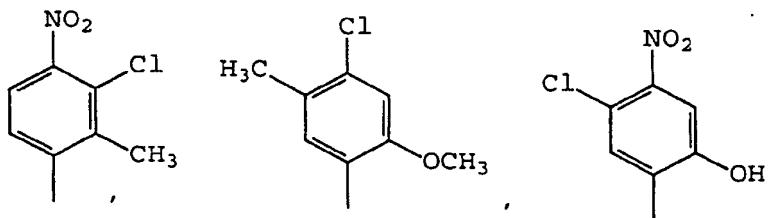
-8-

comprises up to 4 substituents selected from halo,  $OR^3$ ,  $NO_2$ ,  $NH_2$ ,  $N(R^3)_2$ ,  $CO_2R^3$ ,  $CON(R^3)_2$ ,  $COR^3$ ,  $NHCOR^3$ ,  $SO_2NR^3$ ,  $CN$ ,  $SR^3$ , 1,2-methylenoxy, 1,2-ethylenedioxy,  $CF_3$ , ( $C_1-C_6$ )-straight or branched alkyl, ( $C_2-C_6$ )-straight or branched alkenyl or alkynyl.

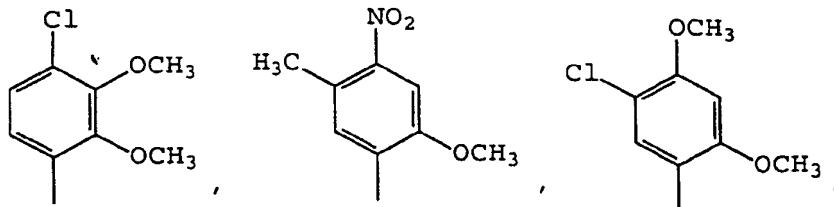
According to a more preferred embodiment,  $Z$  is selected from phenyl or pyridyl, each containing up to 3 substituents selected from halo,  $OR^3$ ,  $NO_2$ ,  $NH_2$ ,  $N(R^3)_2$ ,  $CO_2R^3$ ,  $CON(R^3)_2$ ,  $COR^3$ ,  $NHCOR^3$ ,  $SO_2NR^3$ ,  $CN$ ,  $SR^3$ , 1,2-methylenoxy, 1,2-ethylenedioxy,  $CF_3$  or ( $C_1-C_6$ )-straight or branched alkyl.

According to an even more preferred embodiment,  $Z$  is a 2,4,5-trisubstituted phenyl or a 3,4-disubstituted phenyl, wherein the substituents are selected from halo,  $OR^3$ ,  $NO_2$ ,  $NH_2$ ,  $N(R^3)_2$ ,  $CO_2R^3$ ,  $CON(R^3)_2$ ,  $COR^3$ ,  $NHCOR^3$ ,  $SO_2NR^3$ ,  $CN$ ,  $SR^3$ , 1,2-methylenoxy, 1,2-ethylenedioxy,  $CF_3$  or ( $C_1-C_6$ )-straight or branched alkyl.

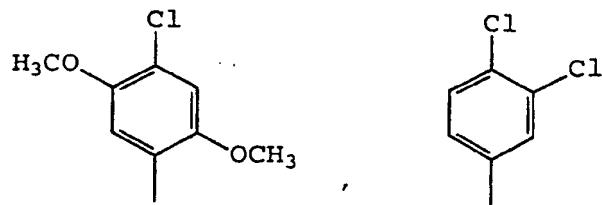
Some specific examples of preferred  $Z$  are:



20



-9-



Most preferred are compounds wherein Z is 4-chloro-2-methyl-5-nitro-phenyl, 4-chloro-2-methoxy-5-methyl-phenyl, 5-chloro-2-hydroxy-4-nitrophenyl, 2,4-dimethoxy-5-chlorophenyl, 2-methoxy-4-nitro-5-methylphenyl, 2,5-dimethoxy-4-chlorophenyl, 3,4-dichlorophenyl.

According to another preferred embodiment, Y is O or S. Most preferably, Y is O.

According to another preferred embodiment, X<sub>1</sub> and X<sub>2</sub> are independently O or NR<sup>2</sup>. More preferably, X<sub>1</sub> and X<sub>2</sub> are both NR<sup>2</sup>. Most preferably, X<sub>1</sub> and X<sub>2</sub> are both NH.

Some specific inhibitors of this invention are set forth in Table 1 below.

TABLE 1

20

compound number	Structure
1	<p>The structure shows a central carbonyl group (C=O) bonded to two amino groups (NH<sub>2</sub>). One amino group is bonded to a phenyl ring (C<sub>6</sub>H<sub>5</sub>), and the other is bonded to a 4,4-dichlorophenyl ring (a benzene ring with two chlorine atoms at the 4 and 4' positions).</p>

-10-

compound number	Structure
2	
3	
4	
5	
6	
7	

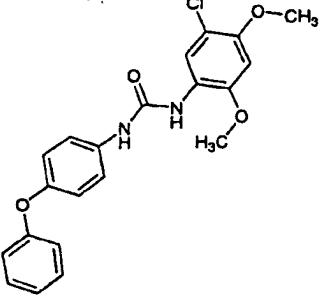
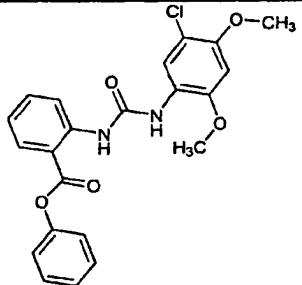
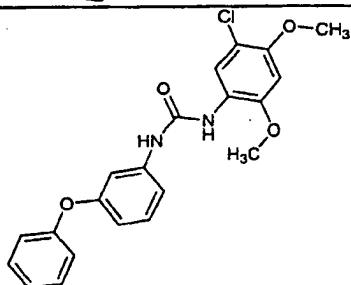
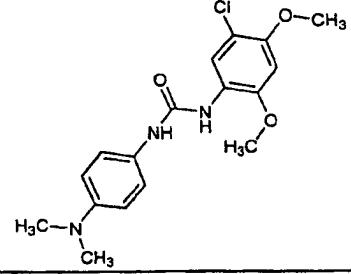
-11-

compound number	Structure
8	
9	
10	
11	
12	
13	

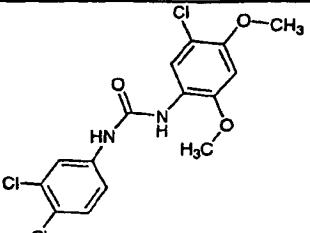
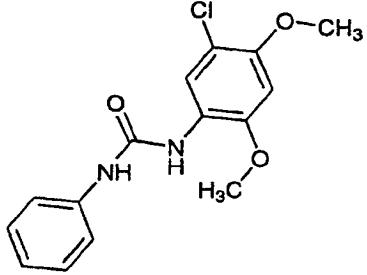
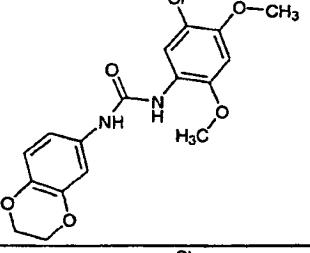
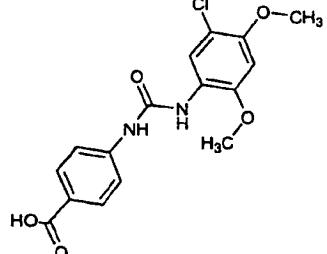
-12-

compound number	Structure
14	
15	
16	
17	
18	
19	

-13-

compound number	Structure
20	
21	
22	
23	

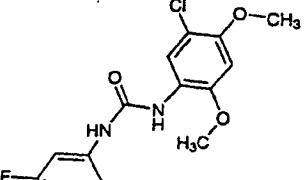
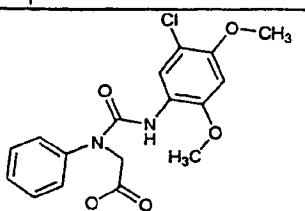
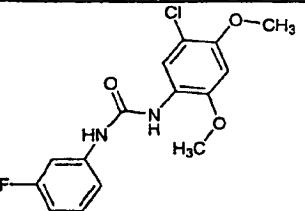
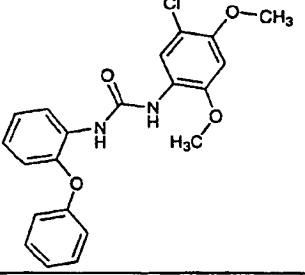
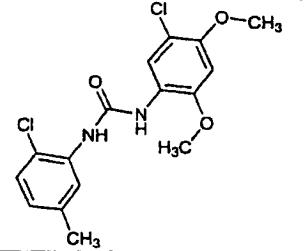
-14-

compound number	Structure
24	
25	
26	
27	

-15-

compound number	Structure
28	
29	
30	
31	
32	

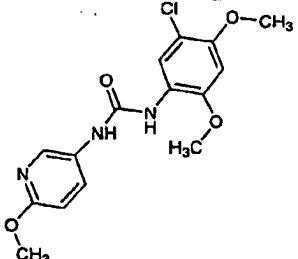
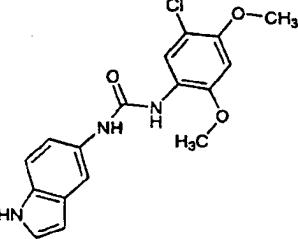
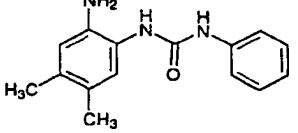
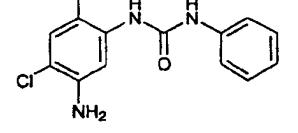
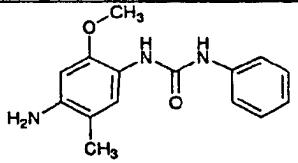
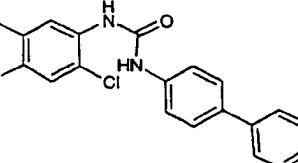
-16-

compound number	Structure
33	
34	
35	
36	
37	

-17-

compound number	Structure
38	
39	
40	
41	

-18-

compound number	Structure
42	
43	
44	
45	
46	
47	

-19-

compound number	Structure
48	
49	
50	
51	
52	
53	

-20-

compound number	Structure
54	
55	
56	
57	
58	
59	

-21-

compound number	Structure
60	
61	
62	
63	
64	
65	

-22-

compound number	Structure
66	
67	
68	
69	
70	

-23-

compound number	Structure
71	
72	
73	
74	
75	

-24-

compound number	Structure
76	
77	
78	
79	
80	

-25-

compound number	Structure
81	
82	
83	
84	
85	

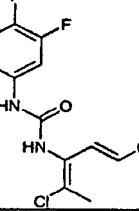
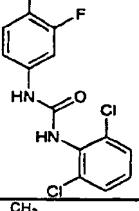
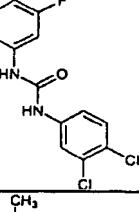
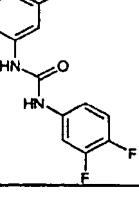
-26-

compound number	Structure
86	
87	
88	
89	
90	

-27-

compound number	Structure
91	
92	
93	
94	
95	

-28-

compound number	Structure
96	
97	
98	
99	
100	

-29-

compound number	Structure
101	
102	
103	
104	
105	

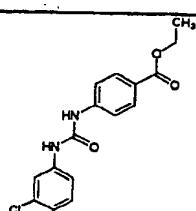
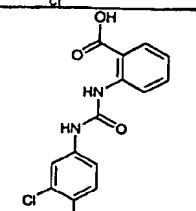
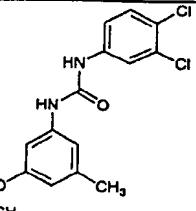
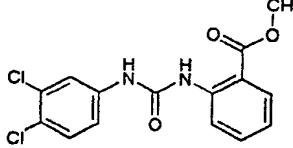
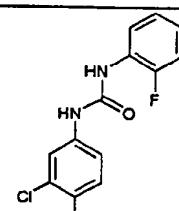
-30-

compound number	Structure
106	
107	
108	
109	
110	

-31-

compound number	Structure
111	
112	
113	
114	
115	

-32-

compound number	Structure
116	
117	
118	
119	
120	

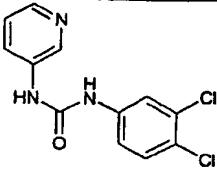
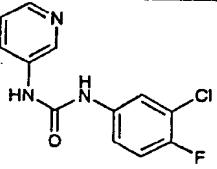
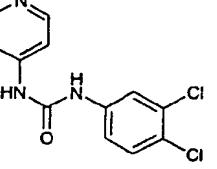
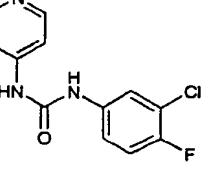
-33-

compound number	Structure
121	
122	
123	
124	
125	

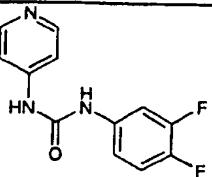
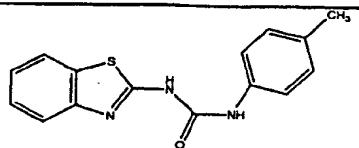
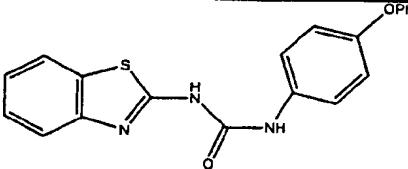
- 3 4 -

compound number	Structure
126	
127	
128	
129	
130	
131	

-35-

compound number	Structure
132	
133	
134	
135	
136	

-36-

compound number	Structure
137	
138	
139	

Preferred compounds of the present invention are compound numbers 3, 4, 6, 12, 13, 22, 24, 25, 29-  
 5 31, 33, 35, 61, 64, 105-107, 114 and 120.

More preferred compounds of the present invention are compound numbers 3, 4, 6, 12, 13, 24, 31, 61, 64, 105 and 107.

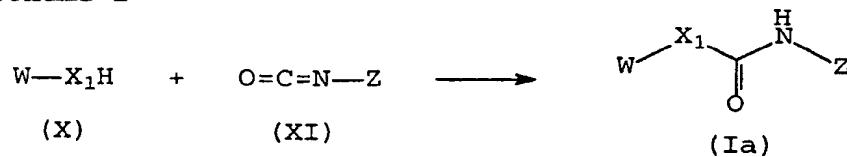
Compounds of formula (I) may be obtained  
 10 using conventional synthetic techniques. Preferably, these compounds are chemically synthesized from readily available starting materials. Modular and convergent methods are also preferred. In a convergent approach, for example, large sections of the final product are  
 15 brought together in the final stages of the synthesis,

-37-

rather than by incremental addition of small pieces to a growing molecular fragment.

Scheme I illustrates a representative example of a convergent process for the synthesis of compounds of formula (Ia), a subset of compounds of formula (I), wherein Y is oxygen and X<sub>2</sub> is NH. The process comprises the reaction of an isocyanate of formula (XI) with an amine, thiol or a hydroxyl compound of formula (X) in a solvent such as methylene chloride. Compounds of formula (I), wherein Y is S or NH can be readily obtained through the process of Scheme 1 by using the thioisocyanate or guanidino analogue of compound of formula (XI), respectively.

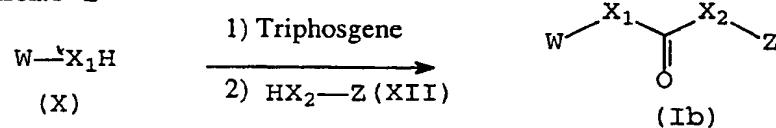
**Scheme 1**



15

Scheme 2 illustrates a representative example of a convergent process for the synthesis of compounds of formula (Ib), a subset of compounds of formula (I), wherein Y is oxygen. A compound of formula (X) is reacted with a coupling reagent such as phosgene, or a phosgene equivalent such as triphosgene, or diethyl carbonate, followed by reaction with a compound of formula (XII) to yield compound of formula (Ib).

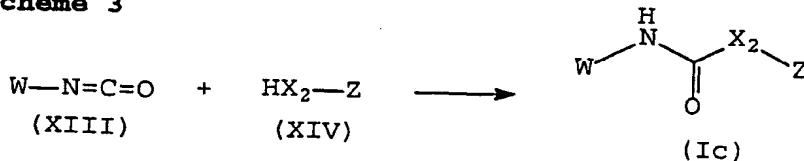
**Scheme 2**



-38-

Scheme 3 illustrates a representative example of a convergent process for the synthesis of compounds of formula (Ic), a subset of compounds of formula (I), wherein Y is oxygen and X<sub>1</sub> is NH.

Scheme 3



The process of Scheme 3 comprises the reaction of an isocyanate of formula (XIII) with an amine, thiol or a hydroxyl compound of formula (XIV), in a solvent such as methylene chloride, to yield compounds of formula (Ic). Compounds of formula (I), wherein Y is S or NH can be readily obtained through the process of Scheme 3 by using the thioisocyanate or guanidino analogue of compound of formula (XIII), respectively.

The activity of the p38 inhibitors of this invention may be assayed *in vitro*, *in vivo* or in a cell line. *In vitro* assays include assays that determine inhibition of either the kinase activity or ATPase activity of activated p38. Alternate *in vitro* assays quantitate the ability of the inhibitor to bind to p38 and may be measured either by radiolabelling the inhibitor prior to binding, isolating the inhibitor/p38 complex and determining the amount of radiolabel bound, or by running a competition experiment where new inhibitors are incubated with p38 bound to known radioligands. These and other useful *in vitro* and cell

-39-

culture assays are well known to those of skill in the art.

Cell culture assays of the inhibitory effect of the compounds of this invention may be used to 5 determine the amounts of TNF, IL-1, IL-6 or IL-8 produced in whole blood or cell fractions thereof in cells treated with inhibitor as compared to cells treated with negative controls. Level of these cytokines may be determined through the use of 10 commercially available ELISAs.

An *in vivo* assay useful for determining the inhibitory activity of the p38 inhibitors of this invention are the suppression of hindpaw edema in rats with *Mycobacterium butyricum*-induced adjuvant 15 arthritis. This is described in J.C. Boehm et al., J. Med. Chem., 39, pp. 3929-37 (1996), the disclosure of which is herein incorporated by reference. The p38 inhibitors of this invention may also be assayed in animal models of arthritis, bone resorption, endotoxin 20 shock and immune function, as described in A. M. Badger et al., J. Pharmacol. Experimental Therapeutics, 279, pp. 1453-61 (1996), the disclosure of which is herein incorporated by reference.

The p38 inhibitors or pharmaceutical salts 25 thereof may be formulated into pharmaceutical compositions for administration to animals or humans. These pharmaceutical compositions, which comprise an amount of p38 inhibitor effective to treat or prevent a p38-mediated condition and a pharmaceutically acceptable carrier, are another embodiment of the 30 present invention. The term "p38-mediated condition" as used herein means any disease or other deleterious condition in which p38 is known to play a role. This includes conditions which are known to be caused by IL- 35 1, TNF, IL-6 or IL-8 overproduction. Such conditions include, without limitation, inflammatory diseases,

-40-

autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, viral disease, and neurodegenerative diseases.

5       Inflammatory diseases which may be treated or prevented include, but are not limited to, acute pancreatitis, chronic pancreatitis, asthma, allergies, and adult respiratory distress syndrome.

10      Autoimmune diseases which may be treated or prevented include, but are not limited to, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, insulin-dependent diabetes mellitus (Type I), autoimmune hemolytic anemia, autoimmune neutropenia, 15     thrombocytopenia, atopic dermatitis, chronic active hepatitis, myasthenia gravis, multiple sclerosis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, psoriasis, or graft vs. host disease.

20      Destructive bone disorders which may be treated or prevented include, but are not limited to, osteoporosis, osteoarthritis and multiple myeloma-related bone disorder.

25      Proliferative diseases which may be treated or prevented include, but are not limited to, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, and multiple myeloma.

30      Infectious diseases which may be treated or prevented include, but are not limited to, sepsis, septic shock, and Shigellosis.

      Viral diseases which may be treated or prevented include, but are not limited to, acute hepatitis infection (including hepatitis A, hepatitis B and hepatitis C), HIV infection and CMV retinitis.

-41-

Degenerative conditions or diseases which may be treated or prevented by the compounds of this invention include, but are not limited to, Alzheimer's disease, Parkinson's disease, cerebral ischemia and 5 other neurodegenerative diseases.

"p38-mediated conditions" also include ischemia/reperfusion in stroke, heart attacks, myocardial ischemia, organ hypoxia, vascular hyperplasia, cardiac hypertrophy and thrombin-induced 10 platelet aggregation.

In addition, p38 inhibitors of this invention are also capable of inhibiting the expression of inducible pro-inflammatory proteins such as prostaglandin endoperoxide synthase-2 (PGHS-2), also 15 referred to as cyclooxygenase-2 (COX-2). Therefore, other "p38-mediated conditions" are edema, analgesia, fever and pain, such as neuromuscular pain, headache, cancer pain, dental pain and arthritis pain.

The conditions and diseases that may be 20 treated or prevented by the p38 inhibitors of this invention may also be conveniently grouped by the cytokine (e.g., IL-1, TNF, IL-6, IL-8) that is believed to be responsible for the disease.

Thus, an IL-1-mediated disease or condition 25 includes rheumatoid arthritis, osteoarthritis, stroke, endotoxemia and/or toxic shock syndrome, inflammatory reaction induced by endotoxin, inflammatory bowel disease, tuberculosis, atherosclerosis, muscle degeneration, cachexia, psoriatic arthritis, Reiter's 30 syndrome, gout, traumatic arthritis, rubella arthritis, acute synovitis, diabetes, pancreatic  $\beta$ -cell disease and Alzheimer's disease.

A TNF-mediated disease or condition includes 35 rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis and other arthritic conditions, sepsis, septic shock, endotoxic shock, gram

-42-

negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, 5 reperfusion injury, graft vs. host reaction, allograft rejections, fever and myalgias due to infection, cachexia secondary to infection, AIDS, ARC or malignancy, keloid formation, scar tissue formation, Crohn's disease, ulcerative colitis or pyrexia. TNF-10 mediated diseases also include viral infections, such as HIV, CMV, influenza and herpes; and veterinary viral infections, such as lentivirus infections, including, but not limited to equine infectious anaemia virus, caprine arthritis virus, visna virus or maedi virus; or 15 retrovirus infections, including feline immunodeficiency virus, bovine immunodeficiency virus, or canine immunodeficiency virus.

IL-8 mediated disease or condition includes diseases characterized by massive neutrophil infiltration, such as psoriasis, inflammatory bowel disease, asthma, cardiac and renal reperfusion injury, adult respiratory distress syndrome, thrombosis and glomerulonephritis.

In addition, the compounds of this infection 25 may be used topically to treat or prevent conditions caused or exacerbated by IL-1 or TNF. Such conditions include inflamed joints, eczema, psoriasis, inflammatory skin conditions such as sunburn, inflammatory eye conditions such as conjunctivitis, 30 pyrexia, pain and other conditions associated with inflammation.

Pharmaceutically acceptable carriers that may be used in these pharmaceutical compositions include, but are not limited to, ion exchangers, alumina, 35 aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as

-43-

phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethyl-cellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, 5 polyethylene glycol and wool fat.

10 The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term 15 "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are 20 administered orally, intraperitoneally or intravenously.

25 Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for 30 example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending 35 medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides.

-44-

Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in 5 their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage 10 forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other 15 dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, 20 capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a 25 capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be 30 added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a 35 suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and

-45-

therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

5 The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including conditions and diseases of the eye, the skin, or the lower intestinal tract. Suitable topical 10 formulations are readily prepared for each of these areas or organs.

15 Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

20 For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, 25 polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not 30 limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

35 For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted

-46-

sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

5       The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, 10 employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

15       The amount of p38 inhibitor that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated the particular mode of administration. Preferably, the compositions should be formulated so that a dosage of between 0.01 - 100 mg/kg body weight/day of the 20 inhibitor can be administered to a patient receiving these compositions.

25       It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The 30 amount of inhibitor will also depend upon the particular compound in the composition.

35       According to another embodiment, the invention provides methods for treating or preventing a p38-mediated condition comprising the step of administering to a patient one of the above-described

-47-

pharmaceutical compositions. The term "patient", as used herein, means an animal, preferably a human.

Preferably, that method is used to treat or prevent a condition selected from inflammatory 5 diseases, autoimmune diseases, destructive bone disorders, proliferative disorders, infectious diseases, degenerative diseases, allergies, reperfusion/ischemia in stroke, heart attacks, organ hypoxia, vascular hyperplasia, cardiac hypertrophy, and 10 thrombin-induced platelet aggregation.

According to another embodiment, the 15 inhibitors of this invention are used to treat or prevent an IL-1, IL-6, IL-8 or TNF-mediated disease or condition. Such conditions are described above.

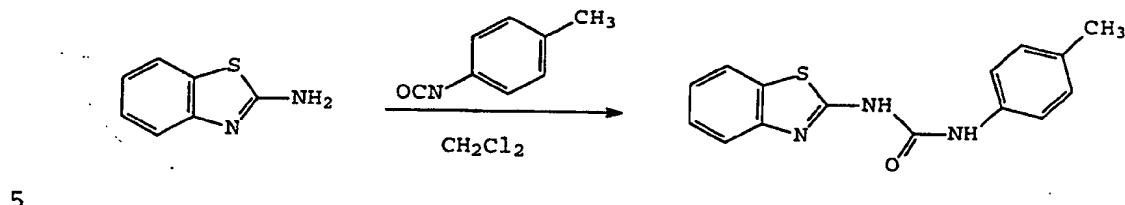
Depending upon the particular p38-mediated 15 condition to be treated or prevented, additional drugs, which are normally administered to treat or prevent that condition may be administered together with the 20 inhibitors of this invention. Those additional agents may be administered separately, as part of a multiple dosage regimen, from the p38 inhibitor-containing composition. Alternatively, those agents may be part 25 of a single dosage form, mixed together with the p38 inhibitor in a single composition.

25 All references cited are herein incorporated by reference.

In order that the invention described herein 30 may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

-48-

EXAMPLE 1  
Synthesis of p38 Inhibitor Compound 138

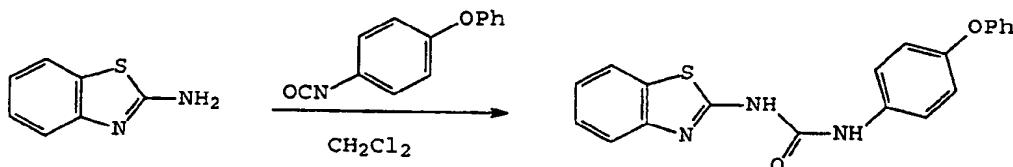


5

2-amino benzothiazole (500 mg, 2.77 mmol) and 4-methylphenylisocyanate (301 uL, 2.77 mmol) were stirred together at room temperature using methylene chloride as a solvent (50 mL). The product from this reaction precipitated from the solvent mixture and was filtered and washed with methylene chloride to yield pure product: 232 mg, 30 % yield. TLC R<sub>f</sub> = 0.55 eluting with 10% methanol in methylene chloride.

15

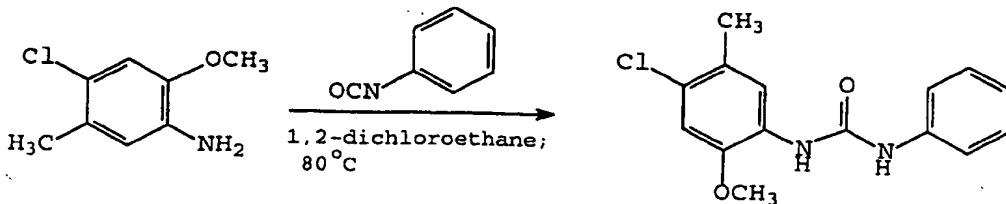
EXAMPLE 2  
Synthesis of p38 Inhibitor Compound 139



20 The same procedure as Example 1 was followed using 4-phenoxyphenylisocyanate. The same scale was used. Pure product was obtained 0.896 mg, 89% yield, R<sub>f</sub> = 0.31 eluting with 10% methanol in methylene chloride.

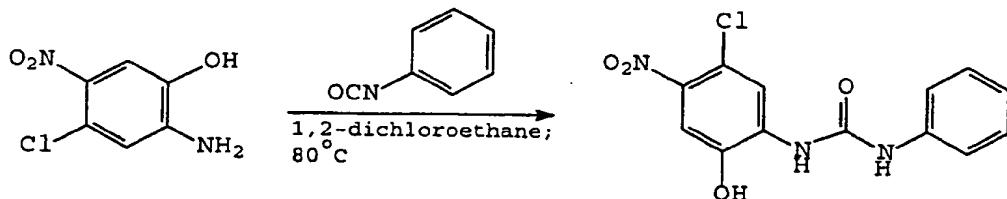
-49-

EXAMPLE 3  
Synthesis of p38 Inhibitor Compound 4



5                   4-chloro-2-methoxy-5-methylaniline (34.3 mg, 0.2 mmol) and a 1M solution of phenylisocyanate in 1,2 dichloroethane (270 ul, 0.27 mmol) were stirred together at 80°C in 1,2 dichloroethane (1 mL). The reaction was heated overnight, then cooled and passed  
10                  through a Varian Bond-Elut SCX cation exchange resin. The filtrate was evaporated in vacuo to yield pure product.

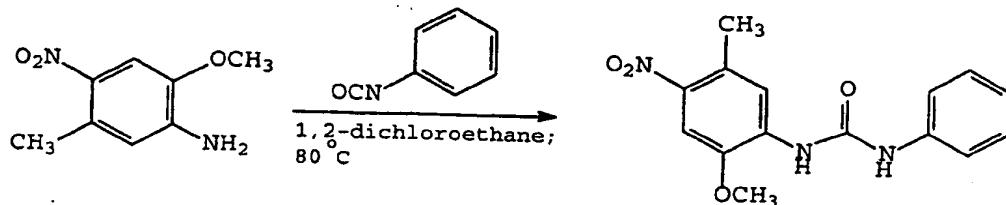
EXAMPLE 4  
Synthesis of p38 Inhibitor Compound 6



15                  2-amino-4-chloro-5-nitrophenol (41.4 mg, 0.22 mmol) and a 1M solution of phenylisocyanate in 1,2 dichloroethane (270 ul, 0.27 mmol) were stirred together at 80°C in 1,2 dichloroethane (1 mL). The reaction was heated overnight, then cooled and passed through a Varian Bond-Elut SCX cation exchange resin. The filtrate was evaporated in vacuo to yield pure product.

-50-

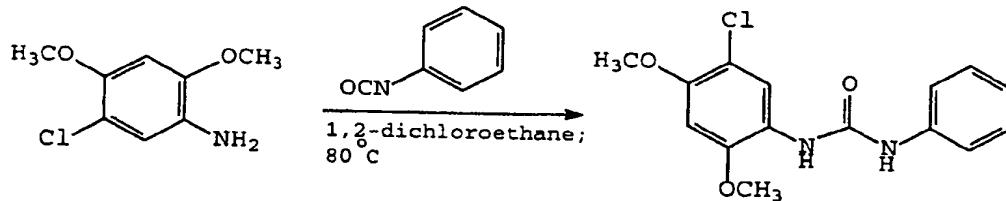
EXAMPLE 5  
Synthesis of p38 Inhibitor Compound 13



5        3-methyl-4-nitro-o-anisidine (37.8 mg, 0.207 mmol) and a 1M solution of phenylisocyanate in 1,2 dichloroethane (270 ul, 0.27 mmol) were stirred together at 80°C in 1,2 dichloroethane (1 mL). The reaction was heated overnight, then cooled and passed through a Varian Bond-Elut SCX cation exchange resin. The filtrate was evaporated in vacuo to yield pure product.

10

15        EXAMPLE 6  
Synthesis of p38 Inhibitor Compound 13



20        5-chloro-2,4-dimethoxyaniline (39.1 mg, 0.208 mmol) and a 1M solution of phenylisocyanate in 1,2 dichloroethane (270 ul, 0.27 mmol) were stirred together at 80°C in 1,2 dichloroethane (1 mL). The reaction was heated overnight, then cooled. Product precipitated from the reaction and was filtered and washed with dichloroethane to yield pure product.

25

-51-

EXAMPLE 7  
Cloning of p38 Kinase in Insect Cells

Two splice variants of human p38 kinase, 5 CSBP1 and CSBP2, have been identified. Specific oligonucleotide primers were used to amplify the coding region of CSBP2 cDNA using a HeLa cell library (Stratagene) as a template. The polymerase chain reaction product was cloned into the pET-15b vector (Novagen). The baculovirus transfer vector, pVL-(His)6-p38 was constructed by subcloning a *Xba*I-*Bam*HI fragment of pET15b-(His)6-p38 into the complementary sites in plasmid pVL1392 (Pharmingen).

10 The plasmid pVL-(His)6-p38 directed the synthesis of a recombinant protein consisting of a 23-residue peptide (MGSSHHHHHHSSGLVPRGSHMLE, where LVPRGS represents a thrombin cleavage site) fused in frame to the N-terminus of p38, as confirmed by DNA sequencing and by N-terminal sequencing of the expressed protein.

15 20 Monolayer culture of *Spodoptera frugiperda* (Sf9) insect cells (ATCC) was maintained in TNM-FH medium (Gibco BRL) supplemented with 10% fetal bovine serum in a T-flask at 27°C. Sf9 cells in log phase were co-transfected with linear viral DNA of *Autographa californica* nuclear polyhedrosis virus (Pharmingen) and transfer vector pVL-(His)6-p38 using Lipofectin (Invitrogen). The individual recombinant baculovirus clones were purified by plaque assay using 1% low melting agarose.

25 30

EXAMPLE 8

Expression And Purification of Recombinant p38 Kinase

35 *Trichoplusia ni* (Tn-368) High-Five™ cells (Invitrogen) were grown in suspension in Excel-405 protein free medium (JRH Bioscience) in a shaker flask at 27°C. Cells at a density of  $1.5 \times 10^6$  cells/ml were

-52-

infected with the recombinant baculovirus described above at a multiplicity of infection of 5. The expression level of recombinant p38 was monitored by immunoblotting using a rabbit anti-p38 antibody (Santa Cruz Biotechnology). The cell mass was harvested 72 hours after infection when the expression level of p38 reached its maximum.

5 Frozen cell paste from cells expressing the (His)<sub>6</sub>-tagged p38 was thawed in 5 volumes of Buffer A (50 mM NaH<sub>2</sub>PO<sub>4</sub> pH 8.0, 200 mM NaCl, 2mM  $\beta$ -Mercaptoethanol, 10% Glycerol and 0.2 mM PMSF). After 10 mechanical disruption of the cells in a Microfluidizer, the lysate was centrifuged at 30,000 x g for 30 minutes. The supernatant was incubated batchwise for 15 3-5 hours at 4°C with Talon™ (Clontech) metal affinity resin at a ratio of 1 ml of resin per 2-4 mgs of expected p38. The resin was settled by centrifugation at 500 x g for 5 minutes and gently washed batchwise 20 with Buffer A. The resin was slurried and poured into a column (approx. 2.6 x 5.0 cm) and washed with Buffer A + 5 mM imidazole.

25 The (His)<sub>6</sub>-p38 was eluted with Buffer A + 100 mM imidazole and subsequently dialyzed overnight at 4°C against 2 liters of Buffer B, (50 mM HEPES, pH 7.5, 25 mM  $\beta$ -glycerophosphate, 5% glycerol, 2mM DTT). The His<sub>6</sub> tag was removed by addition of at 1.5 units thrombin (Calbiochem) per mg of p38 and incubation at 20°C for 2-3 hours. The thrombin was quenched by addition of 0.2 mM PMSF and then the entire sample was loaded onto 30 a 2 ml benzamidine agarose (American International Chemical) column.

35 The flow through fraction was directly loaded onto a 2.6 x 5.0 cm Q-Sepharose (Pharmacia) column previously equilibrated in Buffer B + 0.2 mM PMSF. The p38 was eluted with a 20 column volume linear gradient to 0.6M NaCl in Buffer B. The eluted protein peak was

-53-

pooled and dialyzed overnight at 4°C vs. Buffer C (50 mM HEPES pH 7.5, 5% glycerol, 50 mM NaCl, 2 mM DTT, 0.2 mM PMSF).

5 The dialyzed protein was concentrated in a Centriprep (Amicon) to 3-4 mls and applied to a 2.6 x 100 cm Sephacryl S-100HR (Pharmacia) column. The protein was eluted at a flow rate of 35 mls/hr. The main peak was pooled, adjusted to 20 mM DTT, concentrated to 10-80 mgs/ml and frozen in aliquots at 10 -70°C or used immediately.

EXAMPLE 9

Activation of p38

15 P38 was activated by combining 0.5 mg/ml p38 with 0.005 mg/ml DD-double mutant MKK6 in Buffer B + 10mM MgCl<sub>2</sub>, 2mM ATP, 0.2mM Na<sub>2</sub>VO<sub>4</sub> for 30 minutes at 20°C. The activation mixture was then loaded onto a 1.0 x 10 cm MonoQ column (Pharmacia) and eluted with a linear 20 column volume gradient to 1.0 M NaCl in 20 Buffer B. The activated p38 eluted after the ADP and ATP. The activated p38 peak was pooled and dialyzed against buffer B + 0.2mM Na<sub>2</sub>VO<sub>4</sub> to remove the NaCl. The dialyzed protein was adjusted to 1.1M potassium phosphate by addition of a 4.0M stock solution and 25 loaded onto a 1.0 x 10 cm HIC (Rainin Hydropore) column previously equilibrated in Buffer D (10% glycerol, 20mM  $\beta$ -glycerophosphate, 2.0mM DTT) + 1.1MK<sub>2</sub>HPO<sub>4</sub>. The protein was eluted with a 20 column volume linear gradient to Buffer D + 50mM K<sub>2</sub>HPO<sub>4</sub>. The double 30 phosphorylated p38 eluted as the main peak and was pooled for dialysis against Buffer B + 0.2mM Na<sub>2</sub>VO<sub>4</sub>. The activated p38 was stored at -70°C.

-54-

EXAMPLE 10  
p38 Inhibition Assays

Inhibition of Phosphorylation of EGF Receptor Peptide

5         This assay was carried out in the presence of 10 mM MgCl<sub>2</sub>, 25 mM  $\beta$ -glycerophosphate, 10% glycerol and 100 mM HEPES buffer at pH 7.6. For a typical IC<sub>50</sub> determination, a stock solution was prepared containing 10 all of the above components and activated p38 (5 nM). The stock solution was aliquotted into vials. A fixed volume of DMSO or inhibitor in DMSO (final 15 concentration of DMSO in reaction was 5%) was introduced to each vial, mixed and incubated for 15 minutes at room temperature. EGF receptor peptide, KRELVEPLTPSGEAPNQALLR, a phosphoryl acceptor in p38-catalyzed kinase reaction (1), was added to each vial to a final concentration of 200  $\mu$ M. The kinase 20 reaction was initiated with ATP (100  $\mu$ M) and the vials were incubated at 30°C. After 30 minutes, the reactions 25 were quenched with equal volume of 10% trifluoroacetic acid (TFA).

25         The phosphorylated peptide was quantified by HPLC analysis. Separation of phosphorylated peptide from the unphosphorylated peptide was achieved on a reverse phase column (Deltapak, 5  $\mu$ m, C18 100D, part no. 011795) with a binary gradient of water and acetonitrile, each containing 0.1% TFA. IC<sub>50</sub> (concentration of inhibitor yielding 50% inhibition) 30 was determined by plotting the % activity remaining against inhibitor concentration.

35         The results for several of the inhibitors of this invention are depicted in Table 2 below:

-55-

TABLE 2

Compound	IC <sub>50</sub> (μM)	Compound	IC <sub>50</sub> (μM)
3	0.37	35	0.63
4	0.1	37	5.1
6	0.14	40	1.5
10	4.2	61	0.14
12	0.38	62	5.6
13	0.14	63	5.8
15	5.8	64	0.13
20	8	105	0.5
22	1.9	106	1.82
24	0.5	107	0.1
25	1.0	118	14.7
28	7.4	119	6.3
29	1.45	121	2.2
30	1.2	122	15.1
31	0.5	125	8.4
33	1.82	126	5.6
34	19	131	6.3
35	0.63		

5        Other inhibitors of this invention will also  
 inhibit the kinase activity of p38.

10      While we have hereinbefore presented a number  
 of embodiments of this invention, it is apparent that  
 our basic construction can be altered to provide other  
 embodiments which utilize the methods of this  
 invention.

-56-

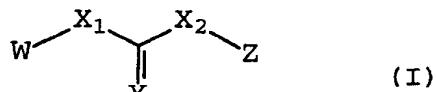
CLAIMS

We claim:

5

1. A compound of the formula:

10



wherein:

15 W is a saturated, partially saturated or aromatic monocyclic or bicyclic ring system optionally comprising up to 4 heteroatoms selected from N, O, and S, wherein W optionally comprises upto 4 substituents independently selected from R<sup>1</sup> and R<sup>4</sup>;

20

wherein R<sup>1</sup> is halogen, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>, CO<sub>2</sub>R<sup>3</sup>, CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-methylenoxy, 1,2-ethylenedioxy or CF<sub>3</sub>;

Y is O, S or NH;

X<sub>1</sub> and X<sub>2</sub> are independently selected from O, S or NR<sup>2</sup>;

25

wherein R<sup>2</sup> is selected from H or C<sub>1</sub>-C<sub>6</sub> straight or branched alkyl, C<sub>2</sub>-C<sub>6</sub> straight or branched alkenyl or alkynyl, wherein R<sup>2</sup> is optionally substituted with -OH, -N(R<sup>3</sup>)<sub>2</sub>, -Z, -CO<sub>2</sub>R<sup>3</sup> or -CO-N(R<sup>3</sup>)<sub>2</sub>;

30

R<sup>3</sup> is selected from H, C<sub>1</sub>-C<sub>6</sub> straight or branched alkyl, C<sub>2</sub>-C<sub>6</sub> straight or branched alkenyl or alkynyl, wherein R<sup>3</sup> is optionally substituted with

-57-

halo, -OH, -OR<sup>4</sup>, -NO<sub>2</sub>, -NH<sub>2</sub>, -N(R<sup>4</sup>)<sub>2</sub>, -CO<sub>2</sub>R<sup>4</sup>, -CO-N(R<sup>4</sup>)<sub>2</sub>, -Z, -CN, -SR<sup>4</sup>, CF<sub>3</sub> or -SO<sub>2</sub>NR<sup>4</sup>;

R<sup>4</sup> is independently H, (C<sub>1</sub>-C<sub>6</sub>)-straight or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl or alkynyl;

Z is selected from C<sub>3</sub>-C<sub>7</sub>-cycloalkyl, C<sub>5</sub>-C<sub>7</sub>-cycloalkenyl or monocyclic or bicyclic, aromatic or non-aromatic ring systems comprising 5-7 members per ring, wherein said ring system optionally comprises up to 4 heteroatoms selected from N, O and S, and wherein Z optionally comprises up to 4 substituents independently selected from R<sup>1</sup> and R<sup>4</sup>.

15 2. The compound according to claim 1, wherein W is an aromatic 5-7 membered monocyclic or bicyclic ring system comprising up to 4 heteroatoms selected from N, O and S, wherein W comprises upto 4 substituents selected from R<sup>1</sup> or R<sup>4</sup>.

20 3. The compound according to claim 2, wherein W is an aromatic 6 membered monocyclic ring comprising upto 2 heteroatoms selected from N, O and S, wherein W comprises up to 4 substituents selected from R<sup>1</sup> or R<sup>4</sup>.

30 4. The compound according to claim 1, wherein W is a phenyl or pyridyl ring optionally comprising upto 3 substituents selected from halo, methyl, methoxy, ethoxy, 1,2-methylenoxy, 1,2-ethylenedioxy, -COOH, -COOCH<sub>3</sub>, or -COOC<sub>2</sub>H<sub>5</sub>.

-58-

5. The compound according to claim 1,  
wherein Z is a monocyclic or bicyclic, aromatic or non-  
aromatic ring system comprising 5-7 members per ring,  
5 wherein said ring system optionally comprises up to 4  
heteroatoms selected from N, O and S, and wherein Z  
optionally comprises up to 4 substituents independently  
selected from halo, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>, CO<sub>2</sub>R<sup>3</sup>,  
CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-  
10 methyleneoxy, 1,2-ethylenedioxy, CF<sub>3</sub>, (C<sub>1</sub>-C<sub>6</sub>)-straight  
or branched alkyl, (C<sub>2</sub>-C<sub>6</sub>)-straight or branched alkenyl  
or alkynyl.

15. The compound according to claim 5,  
wherein Z is phenyl or pyridyl, each containing up to 3  
substituents selected from halo, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>,  
CO<sub>2</sub>R<sup>3</sup>, CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-  
methyleneoxy, 1,2-ethylenedioxy, CF<sub>3</sub> or (C<sub>1</sub>-C<sub>6</sub>)-straight  
or branched alkyl.

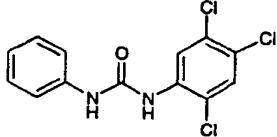
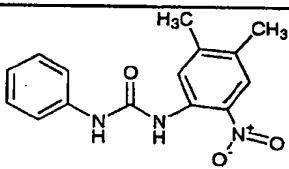
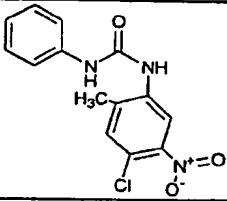
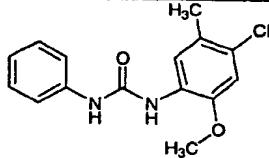
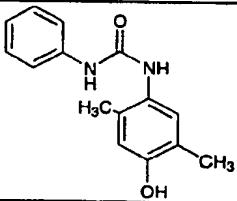
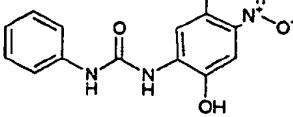
20

7. The compound according to claim 6,  
wherein Z is 2,4,5-trisubstituted phenyl or 3,4-  
disubstituted phenyl, wherein the substituents are  
selected from halo, OR<sup>3</sup>, NO<sub>2</sub>, NH<sub>2</sub>, N(R<sup>3</sup>)<sub>2</sub>, CO<sub>2</sub>R<sup>3</sup>,  
25 CON(R<sup>3</sup>)<sub>2</sub>, COR<sup>3</sup>, NHCOR<sup>3</sup>, SO<sub>2</sub>NR<sup>3</sup>, CN, SR<sup>3</sup>, 1,2-  
methyleneoxy, 1,2-ethylenedioxy, CF<sub>3</sub> or (C<sub>1</sub>-C<sub>6</sub>)-straight  
or branched alkyl.

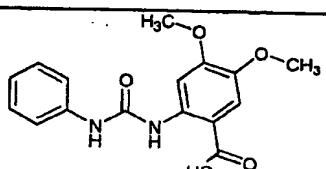
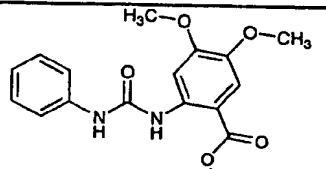
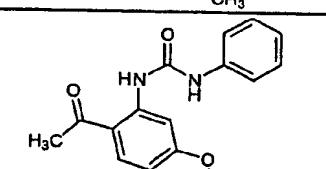
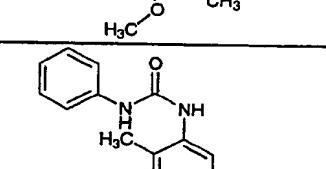
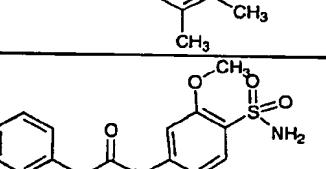
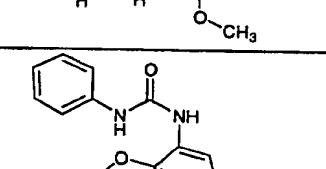
30. The compound according to claim 1,  
wherein the compound is selected from Table 1:

-59-

TABLE 1:

compound number	Structure
1	
2	
3	
4	
5	
6	

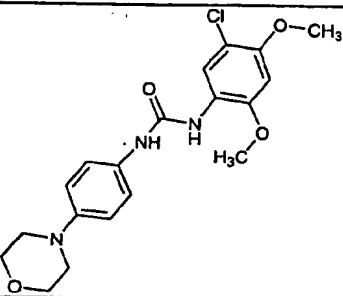
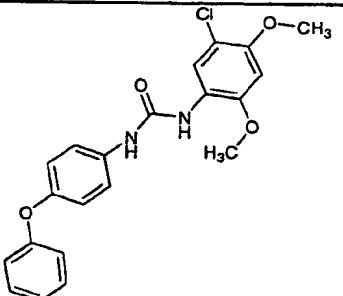
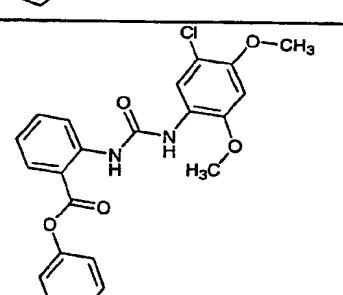
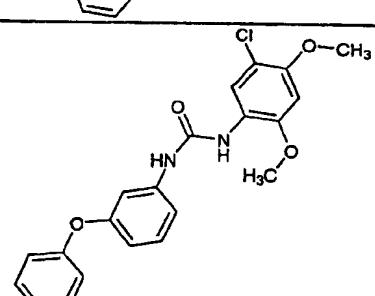
-60-

compound number	Structure
7	
8	
9	
10	
11	
12	

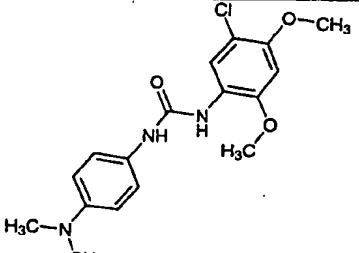
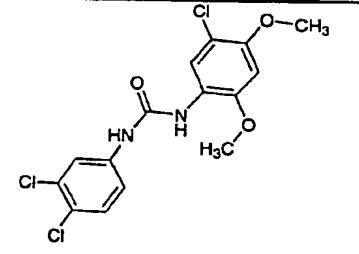
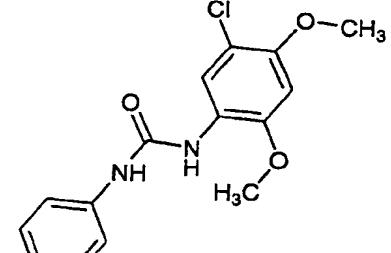
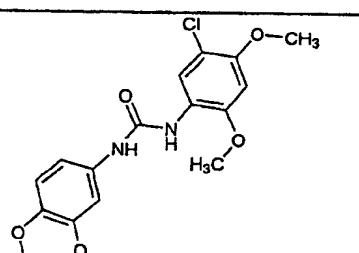
-61-

compound number	Structure
13	
14	
15	
16	
17	
18	

-62-

compound number	Structure
19	
20	
21	
22	

-63-

compound number	Structure
23	
24	
25	
26	

- 64 -

compound number	Structure
27	
28	
29	
30	
31	

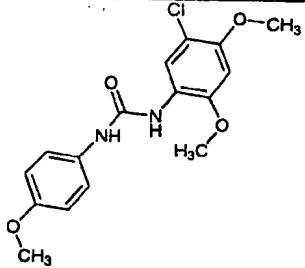
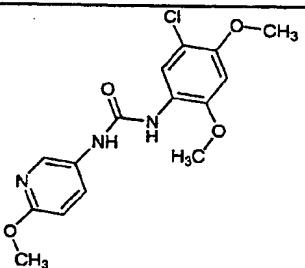
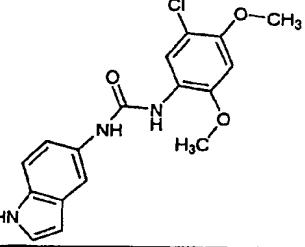
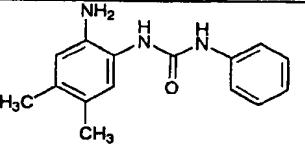
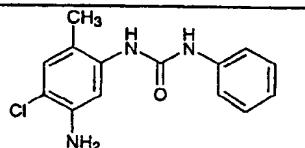
-65-

compound number	Structure
32	
33	
34	
35	
36	

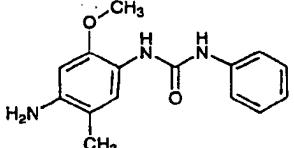
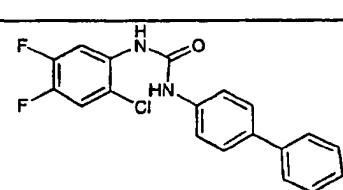
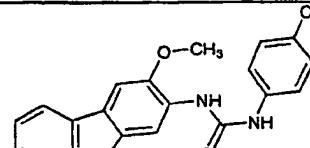
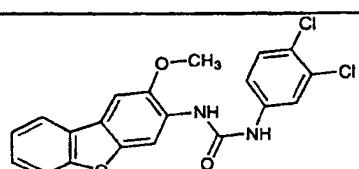
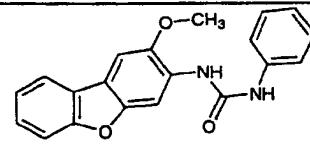
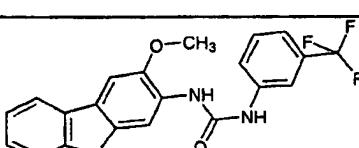
-66-

compound number	Structure
37	
38	
39	
40	

-67-

compound number	Structure
41	
42	
43	
44	
45	

- 68 -

compound number	Structure
46	
47	
48	
49	
50	
51	

-69-

compound number	Structure
52	
53	
54	
55	
56	

-70-

compound number	Structure
57	
58	
59	
60	
61	
62	

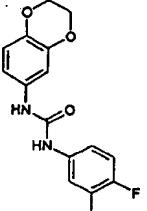
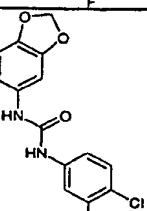
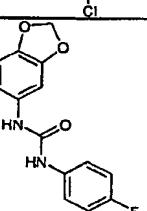
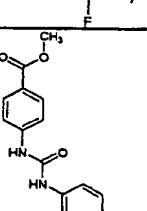
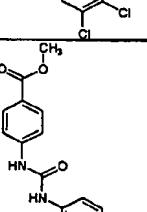
-71-

compound number	Structure
63	
64	
65	
66	
67	

-72-

compound number	Structure
68	
69	
70	
71	
72	

-73-

compound number	Structure
73	
74	
75	
76	
77	

-74-

compound number	Structure
78	
79	
80	
81	
82	

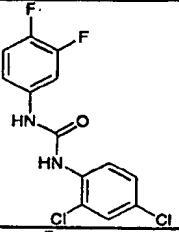
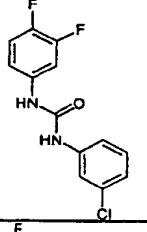
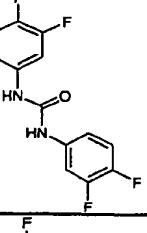
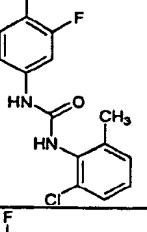
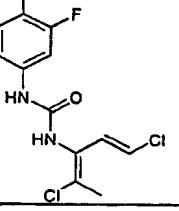
-75-

compound number	Structure
83	
84	
85	
86	
87	

-76-

compound number	Structure
88	
89	
90	
91	
92	

-77-

compound number	Structure
93	
94	
95	
96	
97	

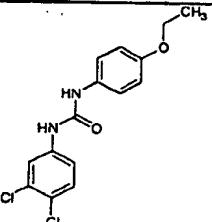
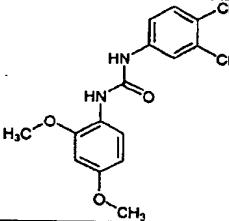
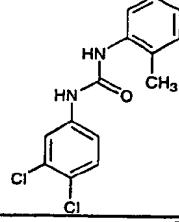
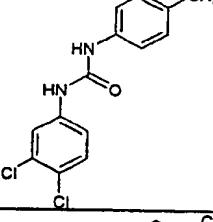
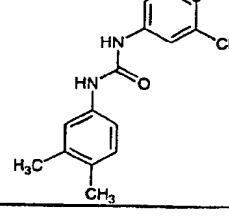
-78-

compound number	Structure
98	
99	
100	
101	
102	

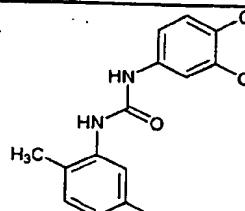
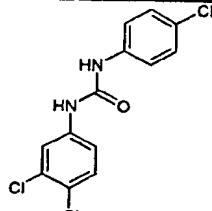
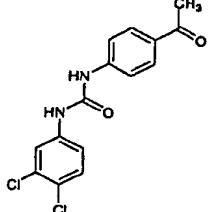
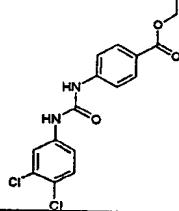
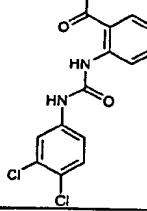
-79-

compound number	Structure
103	
104	
105	
106	
107	

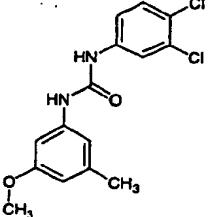
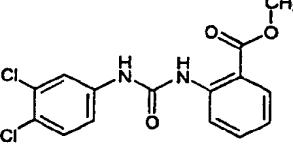
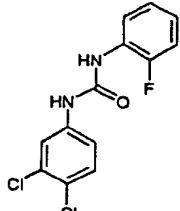
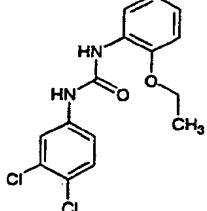
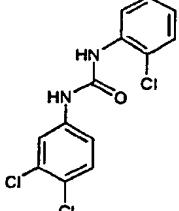
-80-

compound number	Structure
108	
109	
110	
111	
112	

-81-

compound number	Structure
113	
114	
115	
116	
117	

-82-

compound number	Structure
118	
119	
120	
121	
122	

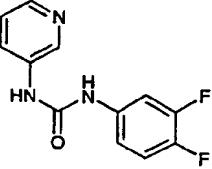
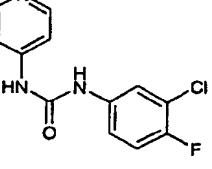
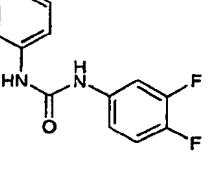
-83-

compound number	Structure
123	
124	
125	
126	
127	

-84-

compound number	Structure
128	
129	
130	
131	
132	
133	

-85-

compound number	Structure
134	
135	
136	
137	

9. A pharmaceutical composition comprising  
 5 an amount of a compound according to any one of claims  
 1 to 8 effective to inhibit p38, and a pharmaceutically  
 acceptable carrier.

10. A method of treating or preventing  
 10 inflammatory disease, autoimmune disease, destructive

-86-

bone disorder, proliferative disorder, infectious disease, viral disease, or neurodegenerative disease in a patient, said method comprising administering to said patient a composition according to claim 9.

5

11. The method according to claim 10, wherein said method is used to treat or prevent an inflammatory disease selected from acute pancreatitis, chronic pancreatitis, asthma, allergies, or adult 10 respiratory distress syndrome.

15

12. The method according to claim 10, wherein said method is used to treat or prevent an autoimmune disease selected from glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Graves' disease, autoimmune gastritis, insulin-dependent diabetes mellitus (Type I), autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, atopic 20 dermatitis, chronic active hepatitis, myasthenia gravis, multiple sclerosis, inflammatory bowel disease, ulcerative colitis, Crohn's disease, psoriasis, or graft vs. host disease.

25

13. The method according to claim 10, wherein said method is used to treat or prevent a destructive bone disorders selected from osteoarthritis, osteoporosis or multiple myeloma-related bone disorder.

30

14. The method according to claim 10, wherein said method is used to treat or prevent a proliferative disease selected from acute myelogenous leukemia, chronic myelogenous leukemia, metastatic 35 melanoma, Kaposi's

-87-

sarcoma, or multiple myeloma.

15. The method according to claim 10,  
wherein  
5 said method is used to treat or prevent an infectious  
disease selected from sepsis, septic shock, or  
Shigellosis.

16. The method according to claim 10,  
10 wherein said method is used to treat or prevent a viral  
disease selected from acute hepatitis infection, HIV  
infection or CMV retinitis.

17. The method according to claim 10,  
15 wherein said method is used to treat or prevent a  
neurodegenerative disease selected from Alzheimer's  
disease, Parkinson's disease or cerebral ischemia.

18. A method of treating or preventing  
20 ischemia/reperfusion in stroke, or myocardial ischemia,  
renal ischemia, heart attacks, organ hypoxia or  
thrombin-induced platelet aggregation in a patient,  
said method comprising the step of administering to  
said patient a pharmaceutical composition according to  
25 claim 9.

19. A method of inhibiting prostaglandin  
endoperoxide synthase-2 in a patient, comprising the  
step of administering to said patient a pharmaceutical  
30 composition according to claim 9.

20. The method according to claim 19,  
wherein said method is used to treat or prevent edema,  
fever, analgesia or to manage pain.

-88-

21. The method according to claim 20,  
wherein said pain is selected from neuromuscular pain,  
headache, cancer pain, dental pain or arthritis pain.

# INTERNATIONAL SEARCH REPORT

Inte. Application No  
PCT/US 98/13496

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C07C275/28 C07D213/06 A61K31/17 A61K31/415

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07C C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 40673 A (SUGEN INC) 19 December 1996 see the whole document	1-7, 9
P, X	WO 97 49399 A (SMITHKLINE BEECHAM CORP ;WIDDOWSON KATHERINE L (US)) 31 December 1997 see the whole document	1-7, 9
P, X	WO 97 49400 A (SMITHKLINE BEECHAM CORP ;WIDDOWSON KATHERINE L (US)) 31 December 1997 see the whole document	1-7, 9
P, X	WO 97 40028 A (VERTEX PHARMA) 30 October 1997 see the whole document	1-7, 9
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance  
"E" earlier document but published on or after the international filing date  
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
"O" document referring to an oral disclosure, use, exhibition or other means  
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.  
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 1998	25/09/1998
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax: (+31-70) 340-3016	Authorized officer  Goetz, G

## INTERNATIONAL SEARCH REPORT

Inter	nal Application No
PCT/US 98/13496	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 31451 A (SMITHKLINE BEECHAM CORP ;ADAMS JERRY LEROY (US); GALLAGHER TIMOTHY) 23 November 1995 cited in the application see the whole document -----	1

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 98/13496

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9640673	A 19-12-1996	US 5773459 A		30-06-1998
		AU 6049396 A		30-12-1996
WO 9749399	A 31-12-1997	NONE		
WO 9749400	A 31-12-1997	NONE		
WO 9740028	A 30-10-1997	AU 2678597 A		12-11-1997
WO 9531451	A 23-11-1995	US 5559137 A		24-09-1996
		JP 10500413 T		13-01-1998